

Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 1 of 11

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FIG._1A



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590 610 ttatatgattatgaccgaaaagatgcagaaggcaagctgcgcgagctt YDYDRKDAE G K L R E 650 630 catctgaaaaagagcattgaagtgatagaggtcccgtctattccagaa LKKSIE VIEVPSI 690 710 cggcatacagttcaccatgaacaaattgaggatttgcttacaacgaca TVHHEQIEDLLTT 750 730 ttgattgaatgcgcttacttttcggtggggaaatggaacttatcagga CAYFSVG K W N LIE 790 70 tcagcaagcttaaagcagcaaaaaccattccttcttatcagtgtgatt ASLKQQKPFLLISVI 830 850 gaaggggagggccgtatgatctctggtgagtatgtctatcctttcaaa S G E Y V Y P F K GEGRM I 890 aaaggagatcatatgttgctgccttacggtcttggagaatttaaactc F.KL K G D H M L L P Y G L G \mathbf{E} 930 gaaggatatgcagaatgtatcgtctcccatctg C·I A E

FIG._1B



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180 NLNEYSE	••	MYKGFTL	09	240 PYAAKTD	: : ELG-KTE		290 ↓↓ GNKVDHA	811 <u>.</u> 1	L9	170		ЕКНТУНН	230
170 EGIIKIRTG	::	OSVVQNG	20	230 RYCRSREKG	: : : EYANIHENGELG-KTE	110	280 290 ↓ GGIEVGPCGNKVDHA	- ;	PGDFFYVPS	160 1	340 ISSFYPVKN	EVIEVPSIP	220
130 140 150 160 170 180 VLNDGDVNIPEYVDWRQKGAVTPVKNQGSCGSCWAFSAVVTIEGIIKIRTGNLNEYSE		PLFFKPVFKERIWGGTALADFGYTIPSQRTGECWAFAAHQNGQSVVQNGMYKGFTL	40	190 200 210 220 240 LLDCDRRSYGCNGGYPWSALQLVAQYGIHYRNTYPYEGVQRYCRSREKGPYAAKTD		100			OCOKDAEIIYGHNATTKEELTTMIERGEWDELLRRVKVKPGDFFYVPSGT	16	300 310 320 330 340 320 330 340 340 340 340 340 340 340 340 34	: AIGKGILALETQQNSDTTYRLYDYDRKDAEGKLRELHLKKSIE VIEVPSIPERHTVHH	210
NOGSCGSCV	. ,	PSQRTGECV	30 T	210 VAQYGIHYRN	: :: : :: : : : : : :	06	260 SIANQPVSVVLEAAGKDFQLYR-	• • • • • • • • • • • • • • • • • • • •	IMIERGEWDI	150	3. IRIKRGIGN	ORKDAEGKLI	200
15(QKGAVTPVF		ALADFGYT	<i>:</i>	10 YPWSALQL	: : FPLLTKIL	80	260 SIANQPVS	••	ATTKEELT'	140	320 STGWGENGYI)TTYRLYDY!	190
140 NIPEYVDWR		FKERIWGGT	20	200 SYGCNGGYI	:	70	250 PYNEGALLY		DAEIIYGHN	130	YILIKNSWC	ALETQQNSI	
130 VLNDGDV		PLFFKPV	10		: : LWEHHRH	7	250 RQVQPYNE	••	YIIDCQK	120	300 AVGYGPN	: AIGKGIL	180
papa_carpa.p	<i>:</i> :			carpa.p			papa_carpa.p_RQVQ		63		_carpa.p		٠.
papa	OE	: YJDE	ગ	papa	GV YJDE	ۯٞ	pape	Ç .	YJDE	□ >	papa	YJDE	į,



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SMYKG	=	SPYKG		Н	KTECW	=======================================	KTECW	ਜ਼ '		AIGKG	= :	ALCKG	્ત	
NGOSANON	- - -	KGPSTVAN	20	110	IHENGELG	= ::	ENEEGELG	110	170	Y YVPSGTVH	=======================================	YVPSGTLH	170	· · · · · · · · · · · · · · · · · · ·
≠ Ecwafaaho		ECWAISAHP	140	100	VHPNDEYAN	=======================================	VHPDDYYAG	100	160	VKVKPGDFF		IKIKPGDFY	160	
SYTIPSORTG	- :::::	SYSIPSESTG	30	06	CDADQDLSVQ	: : :	LDVKEDTSIK	06	150	RGEWDELLRR	= ::	SGDWEGLLRR	150	
VGGTALAD-FO		IGGTALRDRF (20	08	SDRFPLLTKI	:	SDRFPLLTKLI		140	IKEELTTMIEI	:: :: :: ::	SKTELVTMINS	140	
FFKPVFKERIV	=======================================	FLTPVFKEKIV	10	70	нкні ғоді е		SHREVFGGVEC	7.0	130	AEIIYGHNATT		AEIIYGHTAR!	130 L	
MTTEPLE	=	MTQSPIE		09	LSELWER	= = =	LIELWEE	÷	120	IDCOKD	=======================================	IDCKEN		
59 yjde.pep	<u> </u>	- PMI	KT 60	,	19 yjde.pep	I,	 PMI	хт 20	· · · · · ·	yjde.pep	1 -	PMI	ч .	0
	59 yjde.pep MITEPLFFKPVFKERIWGGTALAD-FGYTIPSQRIGECWAFAAHQNGQSVVQNGMYKG	59 yjde.pep mtteplffkpvfkeriwggtalad-fgytipsortgecwafaahongosvvongmykg FT			9	,	9	φ	й — й — н	60 M – M – M – M – M – M – M – M – M – M	120 H L L L L L L L L L L L L L L L L L L	120 H - H - H - H - H - H	120 г – 1 60 д – д	н — н — н — н — н — н — н — н — н — н —

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OTF	EVCOT
FEB	o 5 2004
TRAVET	RADEMARIT

230 2	ннеотергт	: : : STESRKGIT	0	290 2	KGDHMLLPY		KGDHFILPA	e 0		: :	4 · · · · · · · · · · · · · · · · · · ·
220	♥♥ ALETQQNSDTTYRLYDYDRKDAEGKLRELHLKKSIEVIEVPSIPERHTVHHEQIEDLL	: : : : : : : : : :	220 230	280 2	TLIECAYFSVGKWNLSGSASLKQQKPFLLISVIEGEGRMISGEYVYPFKKGDHMLLPY	••	TFVQGEYFSVYKWDINGEAEMAQDESFLICSVIEGSGLLKYEDKTCPLKKGDHFILPA	280 290		1	
210	GKLRELHLKKSIF	GSPRELHFAKAVI	210	270	QOKPFLLISVIEG		ODESFLICSVIEG	270			
190 200	YRLYDYDRKDAE	:	200	260	KWNLSGSASLK		(KWDINGEAEMA(260		CIVSHL	LIVSHI
180	♦♦ ALETQQNSDTT	:	190	240 250	TLIECAYFSVG	=======================================	TFVQGEYFSVY	250	300 310	SFKLEGY: ::	PDFTIKGTCTLIVSHI
	39 yjde.pep	TT PMI	1K 40		99 yjde.pep	GL.	: IMd	E C	· · ·	yjde.pep	PMI

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	MYKG	-	PLAG	Z.	H	TECW	=	TECW	; · ·	, H	IGKG	=	LCKG	·. ·.
20	ZNGQSVVQNG		AHGSSSVKNG	20	110	NIHENGELGK		KLHENGDLGK	110	170	YV FYVPSGTVHA		FYVPSGTLHA	170 TT
40	БЕС МА БААН(GECWAVSAH	T 40	100	7QVHPNDEYA		′ QVНРDDDYA	100	160	RVKVKPGDF	= ::	RIKIKPGDF	160
30	FGYTIPSORT	=======================================	FGYAIPSQKT	30	06	ILDADQDLSV		LLDANMDLSV	06	150	ERGEWDELLR	: : : : : : : : : : : : : : : : : : : :	ESGDWNGLLF	150
20	MTTEPLFFKPVFKERIWGGTALAD-FGYTIPSQRTGECWAFAAHQNGQSVVQNGMYKG		MTHPLFLEPVFKERLWGGTKLRDAFGYAIPSQKTGECWAVSAHAHGSSSVKNGPLAG	20	80	LSELWEHHRHLFGQLEGDRFPLLTKILDADQDLSVQVHPNDEYANIHENGELGKTECW		LDQVWKDHPEIFGFPDGKVFPLLVKLLDANMDLSVQVHPDDDYAKLHENGDLGKTECW	80	140	IDCQKDAEIIYGHNATTKEELTTMIERGEWDELLRRVKVKPGDFFYVPSGTVHAIGKG		IDCKDDAELILGHHASTKEEFKQRIESGDWNGLLRRIKIKPGDFFYVPSGTLHALCKG	140
10	LFFKPVFKER	=======================================	LFLEPVFKER	10	70	ЕННКНГЕСОГ	-	KDHPEIFGFP	. 02	130	DAEI IYGHNA		ОЛЕГІГСННА	130
. :	MTTEP	-	MTHP		09	LSELWI		LDQVWI	09	120	IDCQK		IDCKD	120
	59 yjde.pep	FT	l YDHS	KŢ		19 yjde.pep	Į,	YDHS	Ιλ	ć (/y yjde.pep		YDHS	1

F/G._4A



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		-8-								
8	IEDLL	: : VGNAE		7	MLLPY	<u>-</u>	FILPA		. <i>:</i>	: :
230	кнтуннео:	: :: NHTPEVKE	230	290	үреккерн	:: :: :: :: :: :: :: :: :: :: :: :: ::	YECNAGSH	290		
220	EVIEVPSIPE	: : EVITIPHIDK	220	280	EGRMISGEYV	:: 	SGRIINNGIÇ	280		
210	LRELHLKKSI	:: :: KRTLHIEKAM	210	270	PFLLISVIEG	= = =	TYLLGSVLSG	270		
200	YDRKDAEGK	III : :I: YDRCNDQGQ	200	260	GSASLKQQK	::	GRAAFPSYQ	260		1 1 1 1 1 1
190	♦♦ ALETQQNSDTTYRLYDYDRKDAEGKLRELHLKKSIEVIEVPSIPERHTVHHEQIEDLL	:	1190	250	TLIECAYFSVGKWNLSGSASLKQQKPFLLISVIEGEGRMISGEYVYPFKKGDHMLLPY		VYVQSDYFSVYKWKISGRAAFPSYQTYLLGSVLSGSGRIINNGIQYECNAGSHFILPA	250	0 GEFKLEGYAECIVSHL	: :: GEFTIEGTCEFMISHP 0 310
180	ALETQ	: VLEIQ	180	240	TLIEC	• • • • • •	VYVQS	240	300 GEFKI	
	39 yjde.pep	TT YDHS	II		99 yjde.pep	GL	: XDHS	HF	yjde.pep	YDHS

-1G._4B



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M						F			P				E		L	W	. '
50								70							0		
gga	agg G	ga T		aa K	gct T.	tcg R	tga D	cgc A	r F	tgg G	Cta Y	cgc A	aat: I	acc P	CTC S	aca O	aa
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aaa K									tttc S								g.
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s	v	K	1	N	G	P	L	A	G	K	T	L	D	Q	V	W	
-						21	0						230				
aaa	aga								ggtt								g
K	D	H	•	P	E	I	F	G	F	P	D	G	K	V	F	P	
			25		2			•		27					÷		2
ct	gct	gg	ta	aa	gct	gct	gga	acgo	ccaa	tat	gga	tct	ctc	cgt	gca	agt	CC,
L	L	V	•	K	r	L	D	A	N	M.	D	L	S	V	Q	V	
90	٠.						3	310						33	0		٠.
ca	tco	ctg							aact				atgg	cga	cct	tgg	уt
H	P	D)	D	D	Y	A	K	L	H	E	N	G	D	L	G	
	·	-			350) .	•	٠			3	370					٠.
aạ	aac	gg	ag	tg	ctg	ggta	tat	ca	ttga	ttg	gcaa	aga	atga	cgc	cga	act	ta
K	T	E		С	W	Y	Į	I	D	C	K	D	Ď	Α	E	L	
	3 9	90	•						410)			-		4	130	
		tgg				atgo			caaa			agtt			acç		ca
I	L	G	;	H	Н	Α	S	T	K	E	E	F	K	Q	R	I	
							50						470				
									tgct							agco	ca
E	S	G	}	D	W	N	G	L	L	R	R	I	K	I	K	P	
			49	_						51							5
gg	ag	att	tc						gcgg		cact	cca	atgo	ttt	ato		ag
G	D	F	יז	F	Y	V	P	S	G	T	L	Н	A	L	С	K	
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gg									agca								3C
G	Т	. Т		V	Τ.	F	Ι	0	0	N	S	D	\cdot \mathbf{T}	${f T}$	Y	R	

FIG._5A



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 9 of 11

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610 590 gtatacgattatgaccgctgtaatgaccagggccaaaaaagaactctt V Y D Y D R C N D Q G Q K R T L 650 catatagaaaaagccatggaagtcataacgataccgcatatcgataaa I E K A M E V I T I P H I D K 690 710 gtgcatacaccggaagtaaaagaagttggtaacgctgagatcattgtt V H T P E V K E V G N A E I I V 750 730 tatgtgcaatcagattatttctcagtgtacaaatggaagattagcggc YVOSDYFSVYKWK I S G 70 790 cgagctgcttttccttcatatcaaacctatttgctggggagtgttctg RAAFPSYQTYLLGSVL 830 850 agcggatcaggacgaatcataaataatggtattcagtatgaatgcaat SGSGRI I NNGIQY 870 890 gcaggctcacactttattctgcctgcgcattttggagaatttacaata I L P A H F G E AGSHF 930 gaaggaacatgtgaattcatgatatctcatcct F M I S H P TCE

FIG._5B



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 10 of 11

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	150							170		÷		•			90	
ago	cact	tgt	tgc	aaa												t
S	T	V	A	N	G	P	Y	K,	G	K	T	L	I	E	L	
					21				٠			230				
tgg	gga	aga	gca	ccg	tga	agt	att	cgg	cgg	cgt	aga	aaa			gtt	t
W	E	E	Н	R	E	V	F	G	G	V	E	G	D	R	F	
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				350							70					÷ :
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	39							410							30	:
ato	cat	tta	cgg	gca	tac	ggc	CCC	jctc	aaa	aac	cga	act	tgt	cac	aat	g
I	I	Y	G	Н	T	A	R	S	K	T	E	<u>.</u>	V	${f T}$	M	
					45			. •				470				
at	caa	cag	cgg	gtga	ctg	gga	ggg	jcct	gct	gcg	aag	raat	caa	aat		a
I	N	S	G	D	W	E	G	L	L	R	R	I	K	I	K	
		. 4	90						51	.0						5
CC	ggg	tga	ttt	cta	tta	atgt	gco	gag	cgg	gaac	gct	gca	cgc	att	gtg	C
	G	D	F	Y	Y	V	P	S	G	T	L	Н	A	L	С	
30							550			:			57			
				tgt												С
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FIG._6A



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 11 of 11

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590 610 cgggtgtacgattatgaccgtcttgatagcaacggaagtccgagagag R V Y D Y D R L D S N G S PRE 650 630 cttcattttgccaaagcggtcaatgccgccacggttccccatgtggac AVNAATVPH H F A K 690 710 gggtatatagatgaatcgacagaatcaagaaaaggaataaccattaaa GYIDESTESRKGITIK 750 730 acatttgtccaaggggaatatttttcggtttataaatgggacatcaat SVYKWDI TFVQGEYF 70 790 ggcgaagctgaaatggctcaggatgaatcctttctgatttgcagcgtg G E A E M A Q D E S F L I C S V 830 850 atagaaggaagcggtttgctcaagtatgaggacaaaacatgtccgctc KYEDK EGSGLL 890 870 aaaaaaggtgatcactttattttgccggctcaaatgcccgattttacg K G D H F I L P A Q M P D 930 ataaaaggaacttgtacccttatcgtgtctcatatt TCTLIVSHI I K G

FIG._6B